

## EXAMPLE 2

Mature New Zealand White does were injected with 150 international units of human chorionic gonadotropin (HCG) to induce ovulation, which was expected to occur 10 hr later. Seven hours after treatment with HCG, the does were surgically prepared by injection with Ketamine hydrochloride containing acepromazine and anesthetized under halothane and oxygen. The uterus was exposed by midline incision, and 100  $\mu$ l of sorted or unsorted sperm was placed into the lumen of the anterior tip of each uterine horn through a 21-gauge needle. Standard management practices were used in caring for the rabbits. These does were sacrificed 40 hr post-insemination; uteri were flushed and recovered eggs evaluated. All fertilized eggs recovered were classified as morula. The results of these experiments are shown in Table I.

## EXAMPLE 3

Table II shows the results of inseminations made into the tip of the uterine horn: the number of does that kindled and the phenotypic sex of the offspring compared to the predicted sex. Predicted sex of offspring was based on reanalysis of sorted intact sperm to determine relative DNA content. For reanalysis, the sorted sperm was sonicated for 10 sec and centrifuged at 15,000 g, the supernatant was discarded, and the pellet was resuspended in 9  $\mu$ M bisbenzimidazole H 33342. Phenotypic sex of the offspring was determined soon after birth and confirmed at later ages up to 10 weeks. Recombined X and Y is the sorted X and Y sperm populations recombined immediately before insemination.

## EXAMPLE 4

Using the methods of Examples 1, 2, and 3, viable swine sperm was sorted into viable X and Y chromosome-bearing populations. Two litters (18 pigs) from surgically inseminated boar semen produced 88% females from X-sorted sperm and 67% males from Y-sorted sperm.

It is understood that the foregoing detailed description is given mainly by way of illustration and that modification and variation may be made therein without departure from the spirit and scope of the invention.

I claim:

1. A method for sorting intact, viable, mammalian sperm into X- and Y-chromosome-bearing populations based on DNA content, the method comprising:

- a) staining intact, viable sperm collected from a male mammal with a fluorescent dye capable of selectively staining DNA in living cells by incubating the sperm with the dye at a temperature in the range of about 30°-39° C. for a period of time sufficiently long for staining to take place uniformly but sufficiently short to preserve viability of the sperm;
- b) passing the sperm into an electrically conductive and isotonic viability-supporting sheath fluid to form a suspension of sperm which are caused to flow singly in a stream of sheath fluid;
- c) passing the sheath fluid containing the sperm before an excitation light source causing the stained DNA to fluoresce;
- d) passing the sheath fluid containing the sperm through both a means for detecting the fluorescence of the stained DNA and also a cell sorting means, the means for detecting fluorescence having at least two detectors arranged such that a first

detector determines the orientation of sperm on the basis of magnitude of fluorescence and controls a second detector to measure the DNA content of sperm on the basis of magnitude of fluorescence of those sperm that have been determined to be in a preselected orientation;

- e) selecting by said cell sorting means the sperm having a DNA content corresponding to a desired chromosome which will produce a desired gender of offspring, and separating the selected sperm from nonselected sperm; and
- f) collecting the selected sperm in a viability-supporting collecting fluid.

2. The method of claim 1, wherein said mammal is a rabbit.

3. The method of claim 1, wherein said mammal is a swine.

4. The method of claim 1, wherein said mammal is a bovine.

5. The method of claim 1, wherein said dye is bisbenzimidazole H33342 fluorochrome.

6. The method of claim 1, wherein said incubation is at a temperature of about 39° C. for a period of about 1 hr.

7. The method of claim 1, wherein said incubation is at a temperature of about 35° C. for a period of about 1 hr.

8. The method of claim 1, wherein said incubation is at a temperature of about 30° C. for about 1.5 hr.

9. The method of claim 1, wherein said sheath fluid is phosphate-buffered saline solution, the solution also containing 0.1% bovine serum albumin to enhance sperm viability.

10. The method of claim 1, wherein said collecting fluid is modified test egg yolk extender.

11. The method of claim 1, wherein said sperm are hydrodynamically oriented in the flow of sheath fluid prior to being passed before said light source.

12. The method of claim 1, wherein said sperm are hydrodynamically oriented in the flow of sheath fluid by passing the fluid in a narrow stream through and out of a bevelled injection tip prior to being passed before said light source.

13. A method to preselect the sex of mammalian offspring comprising:

- a) sorting sperm according to the method of claim 1; and
- b) inseminating a female mammal of the same species as the male mammal with the selected sperm in the collecting fluid.

14. A method to preselect the sex of mammalian offspring comprising:

- a) sorting sperm according to the method of claim 1; and
- b) fertilizing an egg obtained from a female mammal of the same species as the male mammal with the selected sperm in the collecting fluid.

15. The method of claim 1, further comprising eliminating sperm which are not properly oriented with an electronic gating system before sorting by said cell sorting means.

16. The method of claim 1, wherein the flow of sperm through the cell sorting means is regulated by an ultrasonic transducer.

17. The method of claim 1, wherein said sperm are sorted on the basis of X- or Y-chromosome DNA content with about 90% efficiency.